Remarks

The foregoing claim amendments are fully supported by the specification as originally filed, and do not add new matter. In particular, recitation of a modification at a free sulfhydryl group of a cysteine residue within the hinge region of the antibody fragment is supported at least at page 19, lines 6-9, and 15-20. The recitation of modification with one or two nonproteinaceous polymer molecules of at least 20 kD finds support at least in the passage bridging pages 19 and 20, and in the Examples. Since the claim amendments are believed to place the application in *prima facie* condition for allowance, do not require further search or extensive consideration by the Examiner, and, as a minimum, present the invention in better form for consideration on appeal, their entry after final rejection is respectfully requested.

Arguments

1. Claims 1-4, 6-13, and 15-34 have been rejected under the judicially created doctrine of obviousness-type double patenting over claims 1-37 of copending Application No. 09/489,394.

Claims 6, 7, 10-12, 17, 19, and 29 have been cancelled. The rejection of the remaining claims is respectfully traversed. The claims of the present application are directed to conjugates in which an antibody fragment is covalently modified by one or two nonproteinaceous polymer molecules at a free sulfhydryl group of a cysteine residue within its hinge region. In contrast, the claims pending in Application 09/489,394 are drawn to conjugates in which the nonproteinaceous polymer molecule is attached at a site where originally there is an inter-chain disulfide bond between the heavy and light chains of the parental antibody, after disrupting the interchain disulfide bond. The inter-chain disulfide bonds between the antibody heavy and light chains is not in the hinge region of the antibody, and the sulfhydryl group participating in this linkage is not "free," therefore, the structures claimed in the present application are clearly different from the structures claims in copending Application 09/489,394. The fact that this structural difference is of non-obvious nature is clearly reflected by the positions taken by the Examiner in the parallel application. Accordingly, the Examiner is respectfully requested to reconsider and withdraw the present rejection.

2. Claims 1-4, 6-13, 15-16, 18-24, and 28-34 were rejected under 35 U.S.C. § 103(a) as "being unpatentable" over Faanes *et al.* (U. S. Patent No. 5,695,760 filed on April 24, 1995). Claims 6, 7, 10-12, 19, and 29 have been cancelled. The rejection of the remaining claims is respectfully traversed.

Claims reciting that a PEG molecule is covalently attached to the hinge region of the antibody fragment (claims 17 and 25) were not included in the present rejection. Indeed, Faanes *et al.* do not teach site-directed modification of an antibody fragment with nonproteinaceous polymer molecules, such as PEG. In particular, Faanes *et al.* do not teach or suggest antibody conjugates in which a nonproteinaceous polymer, such as a PEG molecule, is site-specifically conjugated to an antibody fragment via a free cysteine residue in the hinge region. Since upon entry of the foregoing amendments all claims will recite that the antibody fragment is modified at a free sulfhydryl group of a cysteine residue within the hinge region, the Examiner is respectfully requested to reconsider and withdraw the present rejection.

3. Claims 1-4, 6-13, 15-25, and 28-34 have been rejected under 35 U.S.C. § 103(a) "as being unpatentable" over Faanes *et al.,* (U.S. Patent No. 5,695,760, filed 4/24/95) and further in view of Zapata *et al.* (FASEB J. 9:A1476, 1995).

According to the rejection, Faanes *et al.* teaches the use of mPEG molecules up to 40 kD (column 12, lines 61-63). Accordingly, in view of Zapata et al.'s teaching that a higher molecular weight led to extended half-life, "it would be obvious to use a higher MW PEG and as such in view of Faanes *et al.* which teaches 40kD one skill in the art would use a higher MW to get reduced clearance and as such this would increase the apparent MW of the conjugate and the ratio to those recited in the claims."

The cancellation of claims 6, 7, 10-12, 19, and 29 moots their rejection. The rejection of the remaining claims is believed to be misplaced and is respectfully traversed.

The combination of Faanes et al. and Zapata et al. is legally improper

Faanes *et al.* teach the modification of antibodies with PEG, following a solution-based or column-based glycol-modification method, without controlling the site of modification. The

chemistry used in this approach targets surface exposed ε-amino groups of lysines, and other nucleophiles that are reactive with activated PEG molecules bearing electrophile groups. This procedure does not allow control over the number of PEG molecules attached per antibody molecule, or over the exact locations where the PEG modification of the antibody takes place.

In contrast, Zapata *et al.* teach the site-specific attachment of 5kD and 10kD PEG molecules to a sufhydryl group in the hinge region of an antibody Fab' fragment.

Random and site-directed modifications of antibody fragments with PEG molecules are mutually exclusive, and conclusions drawn from results obtained with one approach are not necessarily applicable to the other approach. Indeed, in the case of random conjugation of PEG moieties to an antibody or antibody fragment, the same molecular weight may result from a wide varieties of different scenarios, including varying numbers of PEG molecules of varying sizes, attached at various sites to the antibody or antibody fragment. This is very difference from the controlled conditions of site-specific PEGylation. Accordingly, one of ordinary skill in the art would not be motivated to combine the teachings of Faanes *et al.* and Zapata *et al.*

Even if the combination of Faanes et al. and Zapata et al. were legally proper, it would not make obvious the invention claimed

The cited Zapata *et al.* reference is an Abstract of a poster presentation at a FASEP meeting in 1995. The poster presentation itself has been submitted by applicants with an Information Disclosure Statement, as Reference #109. The Zapata *et al.* poster presentation teaches that the nonspecific clearance of an antibody Fab fragment with a molecular weight of 49 kD can be decreased as much as 6-fold by the site-directed addition of a 10kD PEG moiety. The Zapata *et el.* poster presentation further teaches that as long the effective molecular size is below 70 kD, clearance decreases as molecular weight increases. Citing Knauf, *J. Biol. Chem.* 263:15064-15070 (1988) (Reference # 79 of record), Zapata *et al.* notes that this might not apply to molecular sizes that exceed the glomerular filtration cutuff size of 70 kD.

Indeed, Knauf *et al.* teach that the *in vivo* clearance rate of an PEG-rIL conjugate rapidly decreases as the effective molecular size increases from 21 to approximately 70 kD, but above 70kD clearance decreases much more slowly. Knauf *et al.* observed no further decrease in clearance rates when the apparent molecule weight of the protein was increased above 200 kD. In view of this teaching, one could not extrapolate from Zapata *et al.*'s data, which were obtained

with less than 70kD antibody-PEG conjugates, that the clearance of antibody-PEG conjugates with an apparent molecular weight of at least about 500 kD would be reduced significantly. A person skilled in the art would not be motivated, based on the Zapata *et al.* poster presentation, and further in view of Knauf *et al.* to make and use antibody-PEG conjugates with apparent molecular weights of at least about 500 kD. Such increase of molecular weight would offer no further benefits over conjugates of smaller size, which are below the glomerular cutoff size.

Accordingly, the Examiner is respectfully requested to reconsider and withdraw the present rejection.

4. Claims 1 and 33-34 were rejected under 35 U.S.C. § 103(a) as "being unpatentable" over Faanes *et al.* and further in view of Harlow *et al.* Faanes *et al.* was cited as in the previous rejection, and Harlow *et al.* was cited for its teaching of nonproteinaceous labels, which "would be obvious to conjugate to antibodies for detection."

In response to the previous rejections, it has been shown that Faanes *et al.* alone, or in combination with Zapata *et al*, does not make obvious the conjugates claimed in the present application. Since the conjugates themselves are patentable, conjugates having nonproteinaceous labels attached to them do not need to show independent indicia of patentability. Accordingly, the Examiner is respectfully requested to withdraw the present rejection.

5. Claims 1, 26 and 27 were rejected under 35 U.S.C. § 103(a) as "unpatentable" over Faanes *et al.* "as applied to claim 1" and further in view of Doerschuk *et al.*. The secondary reference was cited for its teaching of an IL-8 antibody.

Since the claims now recite cite-specific modification of an antibody fragment, which is clearly distinguished over Faanes *et al.*, IL-8 antibody fragments site-specifically modified by one or two PEG molecules are currently claimed are not made obvious by the combination of references on which the present rejection is based. Indeed, claims which recited site-specific modifications within the hinge region were not rejected as obvious over Faanes *et al.* anti-IL-8 antibody fragments with similar site-specific modifications are unobvious for the same reasons.

Attached hereto is a marked-up version of the changes made to the claims by the foregoing amendment. The attached sheet is labeled "**Version with markings to show changes made**."

All claims pending in this application are believed to be in *prima facie* condition for allowance, and an early action to that effect is respectfully solicited.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: November 14, 2002 By: _____

Ginger R. Dreger
Registration No. 33,055
Attorney of Record
Customer No. 20,995
(415) 954-4114

Version with markings to show changes made

Claims 6, 7, 10-12, 17, 19, 29, and 35-52 have been cancelled.

Claims 1, 2, 3, 4, 8, 13, 16, 23, 27, 32, and 33 have been amended as follows:

1. (Twice Amended) A conjugate consisting essentially of [one or more] an antibody [fragments] fragment covalently [attached to] modified by one or [more] two nonproteinaceous polymer molecules at a free sulfhydryl group of a cysteine residue within the hinge region of the antibody fragment, wherein (a) the apparent [size] molecular weight of the conjugate, as determined by size exclusion chromatography, is [(a)] at least about 500 kD, and (b) [at least 8 fold greater than the apparent size of the antibody fragment] the average actual molecular weight of each nonproteinaceous polymer molecule is at least 20 kD.

2. (Amended) The conjugate of Claim 1, wherein the apparent [size] molecular weight of the conjugate is at least about 800 kD.

3. (Amended) The conjugate of Claim 1, wherein the apparent [size] molecular weight of the conjugate is at least about 1,400 kD.

4. (Amended) The conjugate of Claim 1, wherein the apparent [size] molecular weight of the conjugate is at least about 1,800 kD.

8. (Amended) The conjugate of Claim 1, wherein [the conjugate contains no more than one antibody fragment, and wherein] the antibody fragment is selected from the group consisting of Fab, Fab', Fab'-SH, Fv, scFv and $F(ab')_2$.

13. (Amended) The conjugate of Claim 1, wherein the antibody fragment is [attached to more than 2] modified by one nonproteinaceous polymer [molecules] molecule.

16. (Amended) The conjugate of Claim 15, wherein the antibody fragment is [covalently attached to covalently attached to no more than 1] modified by one nonproteinaceous polymer molecule.

23. (Amended) The conjugate of Claim 19, wherein [the conjugate contains no more than one antibody fragment, and wherein] the antibody fragment is a $F(ab')_2$ [and is covalently attached to no more than about 2] modified by two PEG molecules.

27. (Amended) The conjugate of Claim 26, wherein [the conjugate contains no more than one antibody fragment, wherein] the antibody fragment is selected from the group consisting of Fab, Fab' and Fab'-SH[, wherein the antibody fragment is covalently attached to no

more than one nonproteinceous polymer molecule, and wherein the nonproteinaceous polymer molecule is a polyethylene glycol] modified by one PEG molecule having an average actual molecular weight of about 30 kD.

32. (Twice Amended) A conjugate formed by an antibody fragment covalently [attached to] modified by one or [more] two nonproteinaceous polymer molecules at a free sulfhydryl group of a cysteine residue within the hinge region of the antibody fragment, wherein (a) the apparent [size] molecular weight of the conjugate, as determined by size exclusion chromatography, is at least about 500 kD, and (b) the average actual molecular weight of each nonproteinaceous polymer molecule is at least 20 kD, and wherein the molecular structure of the conjugate is free of other matter.

33. (Twice Amended) A conjugate formed by [one or more] an antibody [fragments] fragment covalently [attached to] modified by one or [more] two nonproteinaceous polymer molecules at a free sulfhydryl group of a cysteine residue within the hinge region of the antibody fragment, wherein (a) the apparent molecular weight of the conjugate, as determined by size exclusion chromatography, is at least about 500 kD, (b) the average actual molecular weight of each nonproteinaceous polymer molecule is at least 20 kD, and (c) the antibody fragment incorporates a nonproteinaceous label free of any polymer, and wherein the molecular structure of the conjugate is free of other matter.

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